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Management of wilt of linseed caused by *Fusarium oxysporum* f.sp. *lini* by using fungicides and bioagents

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lini* in linseed (*Linum usitatissimum* L.) is a major constraint responsible for low production and productivity. The crop losses due to wilt in the range of 80-100% have been reported under favourable conditions. An integrated disease management approach is one of the most effective strategies for controlling plant diseases through the combined use of fungicides and biological control agents. In the present study, different fungicides and bioagents were evaluated *in-vivo* under natural field conditions to assess their efficacy. The analysis revealed that, among the eight treatments evaluated on the susceptible linseed variety PKV NL-260 under natural soil conditions seed treatment with *Trichoderma* along with soil application of *Trichoderma* through FYM was the most effective with the lowest wilt incidence (14.59%), 59.82% reduction over the control, highest seed yield (690 kg ha⁻¹) with 77% increase over the control followed by seed treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS which recorded a wilt incidence of 14.93% and both treatments were significantly superior over control which recorded highest wilt incidence 36.32% and lowest yield 390 kg/ha. Four potential bioagents *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in-vitro* by dual culture technique. Among them *Trichoderma harzianum* was the most efficient in inhibiting the growth of *Fusarium oxysporum* f.sp. *lini*. Also six fungicides were evaluated by poison food technique in *in-vitro* conditions for their efficacy. Fungicides which restricted 100% mycelial growth of the *Fusarium oxysporum* f.sp. *lini* were Propiconazole 25% EC, Thiophanate methyl 45% + Pyraclostrobin 5% FS and Captan 70% + Hexaconazole 5% WP.

Keywords: Wilt of linseed, *in-vitro* wilt management, *in-vivo* wilt management, poison food technique, dual culture

Introduction

The linseed (*Linum usitatissimum* L.) is an important rabi oilseed crop which belongs to family Linaceae and genus *Linum*. The oil percent in seed varies from 37.8 to 43% and contain a high level of protein 20.00 to 24.8% and flaxseed oil contains more than 50% linolenic acid, about 80 per cent of the oil produced goes to industries, used as drying oil for the manufacture of many products, including paints, varnishes, linoleum, oilcloth, printer's ink, patent and imitation leather products and many others. Flax fibers are amongst the oldest fiber crops in the world after silk (Narayan, 1987). Linseed oil containing a mixture of rich fatty acids, is rich in two essential fatty acids, alpha-linolenic acid (ALA) and linoleic acid (LA) and is used on food applications where stability is essential (Green *et al.* 1981). In the 2023-24, linseed cultivation covered approximately 2.39 lakh hectares, yielding about 1.67 lakh tonnes, with an average productivity of 698 kg/ha. Madhya Pradesh, Jharkhand and Uttar Pradesh were the leading states in both area and output, jointly contributing nearly 80% to national production. Notably, Rajasthan recorded the highest productivity, followed by Bihar and Nagaland. Among the fungal diseases of linseed, wilt is a major constraint responsible for low production and productivity along with rust and powdery mildew (Kishore *et al.* 2011). The crop losses due to wilt in the range of 80-100% have been reported by (Sattar and Hafiz 1952). To date, various strategies have been developed for the management of plant diseases. Among these, the use of biological control agents has emerged as an effective and eco-friendly approach for managing diseases in a wide range of crops. Among the various bioagents *Trichoderma* spp. have been recognized as highly

effective biological control agents that successfully suppress numerous plant pathogens (Jain *et al.*, 2017; Kharte *et al.*, 2022). Fungicides are commonly used as seed treatments for the management of seed-borne diseases because they effectively eradicate seed-borne inoculum, thereby reducing the primary source of infection and improving seed germination and seedling establishment. The purpose of this study was to determine the most efficient biocontrol and fungicides that are widely utilized in disease control.

Materials and Methods

This experiment was conducted at field of AICRP on linseed and Mustard, College of Agriculture, Nagpur during *rabi* season 2024-25 and laboratory of Plant Pathology Section, College of Agriculture, Nagpur.

In-vivo management of linseed wilt under sick field conditions: Eight treatments comprising of different bioagents and fungicides were evaluated against *Fusarium*

oxysporum f.sp. *lini* under natural field condition. Untreated seeds were used as control. Susceptible linseed variety PKV NL-260 was used in the experiment. Each treatment was plotted in plot size 5 x 4 m² with four replications in Randomized Block Design (RBD). At each treatment, observations on total number of linseed plants and total wilted plants in 1m² area were recorded to calculate per cent disease incidence of wilt by using formula as detailed below.

$$\text{Percent Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

The observations were recorded regarding the per cent disease incidence at 30, 45, 60 DAS, per cent disease reduction over control, yield/plot and percent yield increase over control. Treatment details are as follows.

Table 1: Treatment details of field experiment

Tr. No.	Treatment Details	Rate of Application
T ₁	Seed treatment with Carbendazim 50% + Mancozeb 50% WP	4 g/kg of seed
T ₂	Seed treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS	2 ml/kg of seed
T ₃	Seed treatment with Carbendazim 50% WP	2 g/kg seed
T ₄	Seed treatment with Carboxin 37.5% + Thiram 37.5% DS	3 g/kg seed
T ₅	Seed treatment with <i>Trichoderma</i> and soil application of <i>Trichoderma</i>	4 g/kg of seed + 2.5 kg/250 kg FYM/ha
T ₆	Seed treatment with native <i>Trichoderma viride</i>	4 g/kg seed
T ₇	Seed treatment with native isolates of <i>Azotobacter</i> + PSB + <i>Trichoderma</i>	4 g/kg seed
T ₈	Control	

In-vitro evaluation of bio agents against *Fusarium oxysporum* f.sp. *lini* by dual culture technique

Four bioagents *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* were evaluated against *Fusarium oxysporum* f.sp. *lini* by dual culture technique. In case of *Trichoderma* spp. 5 mm discs of actively growing culture of pathogen and antagonists were placed at opposite sides on PDA media at distance of 5 cm. In case of *Pseudomonas fluorescens* and *Bacillus subtilis* 5 mm diameter mycelial disc from one week old test fungus was cut aseptically with the help of cork borer and placed in centre of petri plates containing a mixture of sterilized PDA and NA medium and bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* were streaked in a square shape around the centrally placed pathogen. The plates containing PDA medium inoculated

with pathogen alone served as control. All plates were incubated at 28 ± 2°C for seven days and all treatments were replicates four times. After the incubation period, the radial growth of the fungal mycelium on each plate was measured. The percent inhibition of radial growth of the pathogen by bio agents in comparison to the control was calculated using the formula described by Vincent (1927).

$$\text{Per cent Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where;

- C = Growth of fungal mycelium in control.
- T = Growth of fungal mycelium in treatment

Table 2: Treatment details for *in-vitro* evaluation of bio agents

Tr. No.	Treatment Details
T ₁	<i>Trichoderma viride</i>
T ₂	<i>Trichoderma harzianum</i>
T ₃	<i>Pseudomonas fluorescens</i>
T ₄	<i>Bacillus subtilis</i>
T ₅	Control

In-vitro evaluation of fungicides against *Fusarium oxysporum* f.sp. *lini* by poison food technique

Seven fungicides were evaluated *in-vitro* against *Fusarium oxysporum* f.sp. *lini* by poison food technique. The requisite quantity of each fungicide was added aseptically to the molten PDA to obtain the desired concentration. About 20 ml of the poisoned medium was poured into each sterilized petri plate and each petri plate was inoculated aseptically at

the centre with a 5 mm diameter mycelial disc cut from the actively growing margin of a seven day old culture of *Fusarium oxysporum* f. sp. *lini*. Control plates containing PDA medium without any fungicide were similarly inoculated with the pathogen. All plates were incubated at 28 ± 2°C for seven days and all treatments were replicated thrice.

Table 3: Treatment details for *in-vitro* evaluation of fungicides

Tr. No.	Treatment Details	Conc. (%)
T ₁	Copper Oxychloride 50% WP	0.20
T ₂	Propiconazole 25% EC	0.30
T ₃	Thiophanate methyl 45% + Pyraclostrobin 5% FS	0.20
T ₄	Captan 70% + Hexaconazole 5% WP	0.15
T ₅	Carbendazim 12% + Mancozeb 63% WP	0.25
T ₆	Azoxystrobin 18.2% + Difenoconazole 11.4 w/w SC	0.10
T ₇	Control	

Results and Discussions

In-vivo evaluation of fungicides and bio agents against *Fusarium oxysporum* f.sp. *lini* under natural field conditions

The data presented in (Table 4, Fig. 1, Plate 1, 2, 3) showed that among all eight treatments minimum mean percent disease incidence (30, 60 and 60 DAS) was recorded in treatment (T₅) seed treatment with *Trichoderma* and soil application of *Trichoderma* through FYM (14.59%) with highest percent disease reduction over control (59.82%) followed by treatment (T₂) seed treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS (14.93%) with percent disease reduction over control (58.89%) and both the treatments were significantly superior over control.

Maximum mean per cent disease incidence was recorded in untreated control (T₈) (36.32%).

Effect of fungicides and bioagents on yield of linseed

As the data showed in (Table 4) the maximum yield was recorded in treatment (T₅) seed treatment with *Trichoderma* and soil application of *Trichoderma* through FYM (690 kg/ha) with 77% increase over control followed by treatment (T₂) seed treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS (629 kg/ha) with 61% increase over control and treatment (T₁) seed treatment with Carbendazim 50% + Mancozeb 50% WP (598 kg/ha) with 53% increase over control. Whereas lowest yield was recorded in untreated control (390 kg/ha).

Table 4: Efficacy of bio agents and fungicide on linseed wilt PDI under wilt sick field condition

Treatment		Per cent disease incidence					Seed yield kg/ha	% yield increase over control
		30 DAS	45 DAS	60 DAS	Mean (30, 45 & 60 DAS)	Percent disease reduction over control		
T ₁	Seed treatment with Carbendazim 50% + Mancozeb 50% WP	13.26 (21.34)	16.64 (24.04)	22.47 (28.27)	17.46	51.94	598	53
T ₂	Seed treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS	10.77 (19.13)	13.99 (21.95)	20.03 (26.57)	14.93	58.89	629	61
T ₃	Seed treatment with Carbendazim 50% WP	11.50 (19.78)	22.68 (28.41)	23.81 (29.19)	19.33	46.78	553	42
T ₄	Seed treatment with Carboxin 37.5% + Thiram 37.5% DS	17.40 (24.62)	23.98 (29.16)	32.03 (34.45)	24.47	32.63	483	24
T ₅	Seed treatment with <i>Trichoderma</i> and soil application of <i>Trichoderma</i>	10.59 (18.96)	14.14 (22.07)	19.05 (25.86)	14.59	59.82	690	77
T ₆	Seed treatment with native <i>Trichoderma viride</i>	12.75 (20.88)	19.62 (26.26)	26.23 (30.79)	19.53	46.22	530	36
T ₇	Seed treatment with native isolates of <i>Azotobacter</i> + PSB + <i>Trichoderma</i>	15.25 (22.97)	22.36 (28.16)	29.02 (32.56)	22.21	38.85	513	32
T ₈	Control	22.70 (28.34)	33.78 (35.52)	52.47 (46.40)	36.32	0	390	-
	SE(m)	0.791	0.87	0.66	-	-	28.61	-
	CD (P=0.05)	2.341	2.58	1.97	-	-	84.71	-
	F test	Sig	Sig	Sig			Sig	

*Figures in parenthesis are arc sine transformation

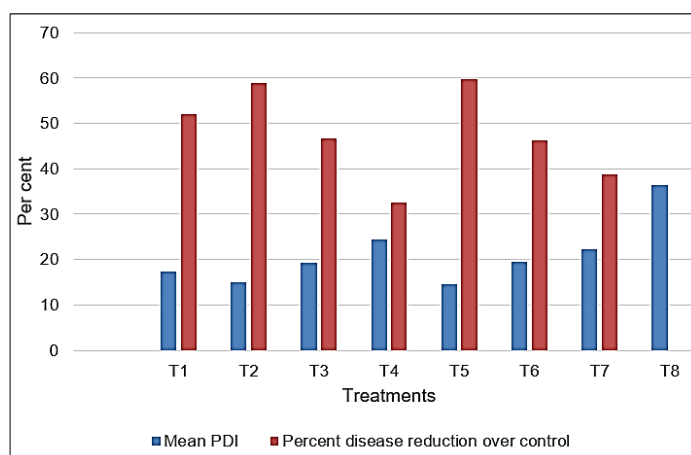
**Fig 1:** Effect of fungicides and bio agents on linseed wilt incidence under sick field condition



Plate 1: General view of experimental field at 60 DAS



Plate 2: T₅- Seed treatment with *Trichoderma* and soil application of *Trichoderma* at 60 DAS



Plate 3: T₈- Control at 60 DAS

***In-vitro* efficacy of bio agents against *Fusarium oxysporum* f.sp. *lini* by dual culture technique:** The data presented in (Table 5, Fig. 2, Plate 4) showed that the

minimum growth of the *Fusarium oxysporum* f.sp. *lini* was recorded by *Trichoderma harzianum* i.e. 15.39 mm followed by *Trichoderma viride* (16.64 mm), *Pseudomonas*

fluorescens (21.14 mm) and *Bacillus subtilis* (23.98 mm). Similarly, maximum per cent growth inhibition was recorded in *Trichoderma harzianum* (82.35) followed by *Trichoderma viride* (80.90%), *Pseudomonas fluorescens* (75.76%) and *Bacillus subtilis* (72.43%).

Similar findings were reported by Brahmanekar *et al.* (2021) who reported, in case of *Fusarium oxysporum* f. sp. *lini*, the

most effective bioagent was *T. harzianum* (86.85%) followed by *T. hamatum* (85.48%), *A. niger* (83.15%), *T. koningii* (81.60%) while *P. fluorescens* (52.04%) was least effective. Similarly, *Trichoderma* spp. was reported as efficient antagonist against several *Fusarium* spp. (Wani *et al.* 2014, Srideepthi and Krishna, 2015, Sudarma *et al.* 2015 and Barari, 2016).

Table 5: In-vitro efficacy of bio agents against mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *lini*

Treatment		<i>Fusarium oxysporum</i> f.sp. <i>lini</i>					
		Mean Colony diameter (mm)			Per cent growth inhibition		
		3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T ₁	<i>Trichoderma viride</i>	11.63	14.50	16.64	66.89	75.52	80.90
T ₂	<i>Trichoderma harzianum</i>	10.50	13.38	15.39	70.11	77.41	82.35
T ₃	<i>Pseudomonas fluorescens</i>	16.00	19.13	21.14	54.45	67.71	75.76
T ₄	<i>Bacillus subtilis</i>	19.25	22.00	23.98	45.21	62.86	72.43
T ₅	Control	35.13	59.25	87.14	-	-	-
	F test	Sig	Sig	Sig			
	S.E ($\pm$ m)	0.27	0.43	0.46			
	C.D (P = 0.01)	1.15	1.79	1.92			

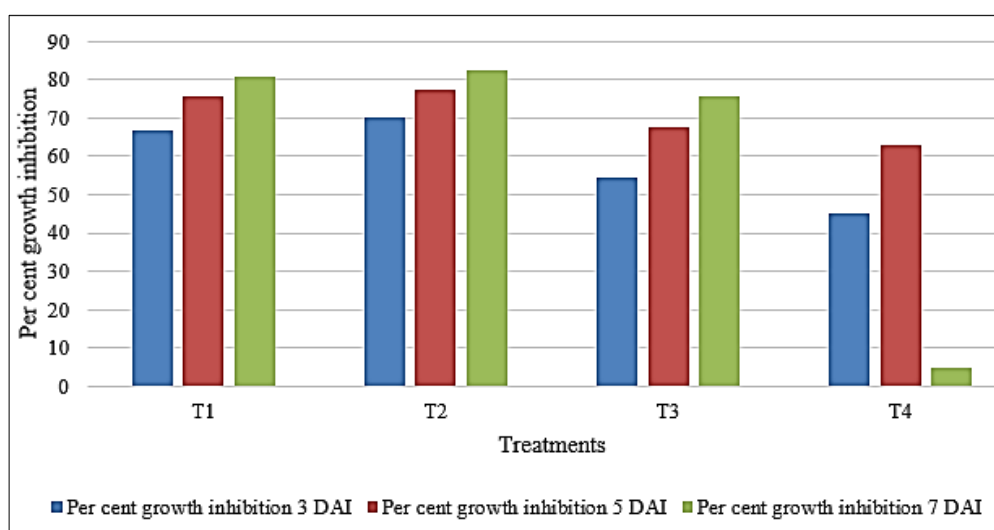


Fig 2: Effect of bio agents on per cent growth inhibition of *Fusarium oxysporum* f.sp. *lini*



Plate 4: In-vitro efficacy of bio agents against *Fusarium oxysporum* f.sp. *lini* on 7th DAI

***In-vitro* efficacy of fungicides against *Fusarium oxysporum* f.sp. *lini* by poisoned food technique**

The data presented in (Table 6, Fig. 3, Plate 5) reveals that among the six fungicides evaluated, the most promising fungicides, which restricted 100% growth of the test pathogen were Propiconazole 25% EC, Thiophanate methyl 45% + Pyraclostrobin 5% FS and Captan 70% + Hexaconazole 5% WP followed by Azoxystrobin 18.2% + Difenconazole 11.4 w/w SC and Carbendazim 12% + Mancozeb 63% WP which recorded 77.12 and 66.27 per cent inhibition, respectively. Whereas the least control of the

pathogen or minimum per cent inhibition was observed in Copper oxychloride 50% WP having 35.71 per cent inhibition.

In a similar way Singh *et al.* (2023) reported that, Propiconazole exhibited cent percent growth inhibition over the control against *Fusarium oxysporum* f.sp. *lini*. The results are also in conformity with the findings of several other workers in case of *F. oxysporum* i.e. Madhavi and Bhattiprolu (2011), Tewari *et al.* (2012), Wani and Nazar (2012) and Bashar and Chakma (2014), Singh *et al.* (2017) and Pimpale *et al.* (2023).

Table 6: *In-vitro* efficacy of fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *lini*

Treatment		Conc. (%)	Fusarium oxysporum f.sp. lini					
			Mean Colony diameter (mm)			Per cent growth inhibition		
			3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T ₁	Copper oxychloride 50% WP	0.20	23.34	39.39	54.32	32.69	33.80	35.71
T ₂	Propiconazole 25% EC	0.30	0.00	0.00	0.00	100.00	100.00	100.00
T ₃	Thiophanate methyl 45% + Pyraclostrobin 5% FS	0.20	0.00	0.00	0.00	100.00	100.00	100.00
T ₄	Captan 70% + Hexaconazole 5% WP	0.15	0.00	0.00	0.00	100.00	100.00	100.00
T ₅	Carbendazim 12% + Mancozeb 63%	0.25	12.82	20.84	28.50	62.99	64.99	66.27
T ₆	Azoxystrobin 18.2% + Difenconazole 11.4 w/w SC	0.10	8.84	14.33	19.33	74.52	75.91	77.12
T ₇	Control	-	34.67	59.50	84.50	-	-	-
	F test		Sig	Sig	Sig			
	S.E (± m)		0.19	0.47	0.63			
	C.D (P = 0.01)		0.83	2.00	2.67			

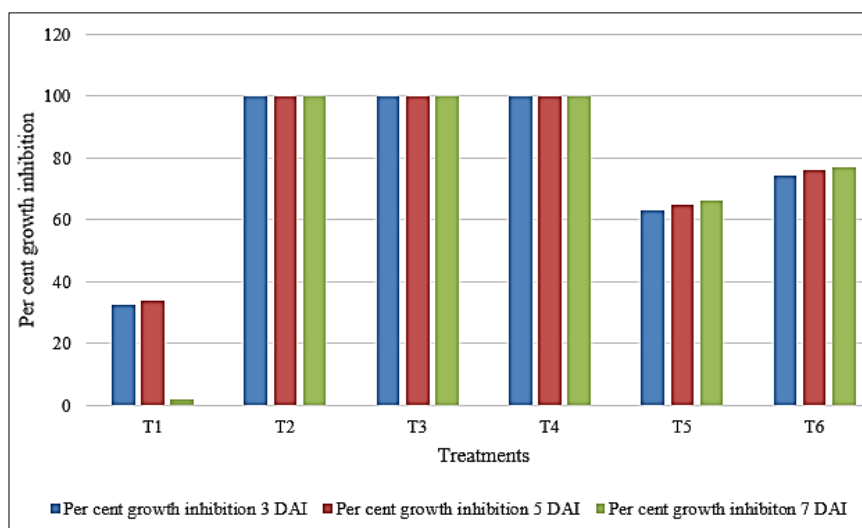


Fig 3: Effect of fungicides on per cent growth inhibition of *Fusarium oxysporum* f.sp. *lini*



Plate 5: *In-vitro* efficacy of fungicides against *Fusarium oxysporum* f.sp. *lini* on 7th DAI

Conclusion

In *in-vivo* evaluation of bio agents and fungicides under sick field condition, seed treatment with *Trichoderma* and soil application of *Trichoderma* found most effective and could be suggested for farmers. *In-vitro* evaluation of bio agents and fungicides, *Trichoderma harzianum* found most efficient and fungicides which showed 100 percent mycelial growth inhibition were Propiconazole 25% EC, Thiophanate methyl 45% + Pyraclostrobin 5% FS and Captan 70% + Hexaconazole 5% WP.

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